Dean&Francis
ISSN 2959-409X

Cancer Treatment Strategy Depending on CRISPR Technology

Tiancong Du [1, *]

[1]Department of Analytical Testing Science, Hong Kong Metropolitan University, Hong Kong, China
[*]Corresponding author: s1337658@live.hkmu.edu.hk

Abstract:

Cancer keeps posing a major threat to human health all over the word. CRISPR technology is an emerging gene editing technique, which offers new ideas for cancer treatment. Compared with traditional therapies including surgery, radiotherapy, and chemotherapy, it has many advantages such as complete cure, high precision, efficiency, and low side effects. The CRISPR system mainly consists of Cas9 and gRNA, of which gRNA helps recognize specific DNA sequences and guides the Cas9 protein to cleavage double-strand. Subsequently, genetic mutations are produced in the process of repair mechanisms. CRISPR therapy for cancer has two main approaches, that are modifying immune cells and editing the genes of cancer cells. This article focused on CRISPR-based CAR-T technology to enhance their ability to recognize and destroy tumor cells. Another strategy directly targets cancer genomes, disabling oncogenes or restoring the function of tumor-suppressor genes. Despite these promising advances, several limitations of CRISPR-based technology remain including off-target effects, reducing editing efficiency, in vivo immune reactions to Cas proteins. Therefore, CRISPR-Cas9 is a versatile and powerful tool for advancing precision oncology, contributing to future clinical applications of more effective, gene-based cancer treatments.

Keywords: CRISPR, Cas9, gRNA, CAR-T, lncRNA

1. Introduction

Cancer is a malignant disease. It seriously endangers human health. The current conventional clinical treatment methods, such as radiotherapy and chemotherapy, have obviously limited problems and side effects. These traditional therapies may cause damage to the patient's healthy tissues and result in very serious complications. Furthermore, traditional therapies are difficult to completely eliminate tumor cells and have a higher risk of recurrence. Cancer is a disease caused by multiple gene mutations. CRISPR technology, as an emerging gene editing technology, can provide a completely new approach for cancer treatment. It is also one of the most mainstream gene editing technologies at present. It can precisely elim-

ISSN 2959-409X

inate genes at specific locations, providing significant assistance for gene editing. It is both precise and efficient [1]. Due to its outstanding capabilities demonstrated, its low cost and convenient operation, it has been applied in multiple fields of cancer treatment and research. This technology is also applied in tumor research and the construction of disease models. However, it still faces problems such as low efficiency and off-target risks at present. This study focused on how CRISPR is applied in cancer treatment theoretically and analyzed it in specific cancer cases. This would provide new insights for future cancer treatment.

2. The Principle and Experimental Applications of CRISPR

2.1 The Origin of CRISPR

According to Hao et al., the CRISPR-Cas9 system originates from bacteria and archaea. It is an immune mechanism that these organisms have evolved to defend against exogenous DNA invasion during their evolution [2]. The CRISPR-Cas9 system can capture invading DNA fragments and degrade them using its own enzymes through double-strand recognition. In 2012, two scientists made innovative viewpoints on the potential of CRISPR-Cas9 in gene editing. And in 2020, the two were awarded the Nobel Prize for their research on it.

2.2 Principle of CRISPR

The main components of the system are the Cas9 protein and gRNA, which is a guide molecule that can recognize and pair with specific DNA sequences. It binds to the Cas9 protein, guiding it to a specific site for cleavage. The Cas9 protein is a type of endonuclease that precisely cuts target DNA fragments. In practice, the gRNA is individually designed based on the sequence of the target gene. The selected target site must be sufficiently specific to avoid CRISPR targeting the wrong site due to close similarity to unrelated DNA fragments. The target gene sequence can be obtained from a reputable gene database.

Niu reported that there is a precursor RNA (crRNA) in CRISPR that can correspond one-to-one with the target gene, which is the basis for accurate identification of the target gene [3]. gRNA is a special RNA processed by crRNA. crRNA completes this process through base complementary pairing and transactivation. This RNA sequence can specifically bind to the target DNA and guide the Cas9 protein to work. The main biological macromolecules of the nuclease Cas9 are the HNH (histidine-asparagine-histidine sequence peptide chain) and RuvC (Repair UV damage-C) domains. These two domains jointly are involved in cutting the DNA chain complementary to the gRNA. Cas9 cleavage creates a double-stranded DNA break. The cell own repair mechanisms can bridge the break and repair the cut fragment [4]. If a foreign gene is to be introduced, a gene vector, usually a modified virus, plasmid, must be introduced into the cell at the same time as the CRISPR system. The vector then integrates the foreign fragment into the cell's DNA after the CRISPR cleavage is complete.

2.3 Application for Engineering of CRISPR

Currently, there are two broad strategies for applying CRISPR to cancer treatment. One is to modify a patient's own T cells to enhance their immune capacity. CAR-T therapy is a prime example. This involves knocking out immune checkpoint genes on T cells, preventing cancer cells from binding to these sites and inhibiting immune escape. The other is to directly edit the cancer cell genome, disrupting genes involved in survival and proliferation, or reactivating tumor suppressor genes.

Table 1. Therapies of CRISPR in cancer

Therapy name	Effects	Process
CAR-T	Enhance the ability of patients' T cells to recognize cancer cells and reduce the immune escape of cancer cells.	T cells are transformed into CAR-T cells mainly through CRISPR technology and combined with the following two methods: first, the PD1 receptor of CAR-T cells is knocked out, or the receptor gene for T cell adenosine is knocked out through CRISPR technology.
CRISPR inhibits expression of oncogenic lncRNA	Reduce or eliminate the expression of oncogenic lncRNA to inhibit the growth of cancer cells.	Use CRISPR to disrupt key segments of a gene that controls lncRNA or delete the entire gene.
CRISPR promotes expression of tumor suppressor lncRNA	Re-express the inactivated tumor suppressor lncRNA in cancer cells and kill cancer cells.	Use CRISPR to promote the transcription of tumor suppressor lncRNA genes, causing cancer cells to re-express inactivated tumor suppressor lncRNAs.

3. Applications of CRISPR in Cancer Therapy

3.1 CAR-T Depending on CRISPR

3.1.1 Principals and process of CAR-T

CAR-T (chimeric antigen receptor T-cell) therapy is a new type of immunotherapy that targets immune evasion of cancer cells. The principle behind CAR-T is to modify T cells through gene editing, making them carry a CAR structure, which is a man-made protein structure that directly recognizes surface antigens on cancer cells. This alters signaling pathways in T cells, changing how it recognizes cancer cells. CAR The edited T cells will synthesize CAR to replace the original cancer cell receptors. It Is reported that traditional CAR-T technology directly inserts the CAR gene into the patient's T cells through viral vectors, which is highly random, while the site-specific recognition of CRISPR technology provides optimization (Table 1) [5].

CRISPR editing also enhances immune responses by altering pre-existing signaling pathways. Specifically, Program death 1 (PD-1) is an inhibitory receptor located on T cells. When bound to the PD-L1 molecule on the cell surface, it prevent T cells from being killed by immune cells. Cancer cells overexpress PD-L1, enabling immune escape. By editing T cell genes and blocking the PD-1-PD-L1 signaling pathway, this immune escape strategy is negated (Table 1). In addition to signaling pathways like PD-1-PD-L1, cancer cells can also accumulate large amounts of adenosine in the hypoxic tumor microenvironment. Adenosine binds to the A2AR adenosine receptor on immune cells, leading to immune escape (Table 1). Knocking out these receptor genes via CRISPR technology enables T cells to accurately and efficiently recognize cancer cells, complementing the function of CAR [6].

Additionally, the CAR-T cells delivered back into the patient's body are derived from the patient's own body, rather than from an external source. This effectively avoids the problem of immune rejection, making therapy safer and more effective.

3.1.2 Application of CAR-T in the treatment of lung cancer

Lung cancer is an immunosuppressive cancer, and the lung cancer microenvironment causes T cells to highly express PD-1 [7]. The disease can be treated with the above-mentioned CAR-T therapy. Researchers selected epidermal growth factor receptor (EGFR) as the target of CAR-T cells. The EGFR-CAR gene is introduced into the edited T cells via CRISPR technology and lentiviral transduction, producing EGFR-CAR protein. CRISPR editing can also inactivate the original PD-1-expressing gene of T cells. Mouse experiments showed that the immune effect of successfully constructed T cells was significantly stronger than that of ordinary T cells and CAR-T cells that were only partially processed [7], indicating that this treatment method has high feasibility and good research prospects, and the two editing strategies have better therapeutic effects.

3.2 CRISPR Editing Important Genes in Cancer

another approach is Using o directly edit the genes of cancer cells using CRISPR technology. Directly removing key genes that promote cancer cell growth can prevent normal proliferation and metastasis. Alternatively, it can repair tumor suppressor genes that are not expressed in cancer cells, correcting pathogenic mutations and restoring their tumor suppressor function. Both approaches can eliminate the root cause of cancer, providing precise and effective treatment.

3.2.1 Knockout of oncogenic lncRNAs via CRISPR

lncRNA (Long non-coding RNA) is a type of signaling molecule with a length of more than 200 nucleotides. It plays an important role in cell growth and differentiation, gene expression regulation. Oncogenic lncRNAs such as HOTAIR, MALAT1 are highly abnormally expressed in cancer cells. They can promote cell division and differentiation, regulate signaling pathways to cause excessive cell proliferation, and promote the metastasis of cancer cells to other tissues in the body. Interfere with or directly knock out the genes that control oncogenic lncRNAs using CRISPR can achieve the effect of inhibiting the growth of cancer cells (Table 1). Under normal conditions, tumor suppressor lncRNAs, such as MEG3, GAS5, inhibit carcinogenesis by regulating cell apoptosis and maintaining normal gene expression. Their dysfunction can cause cells to be unable to normally express tumor suppressor genes, resulting in carcinogenesis [8]. In the tumor microenvironment, the expression level of tumor suppressor lncRNA is significantly reduced. Repair the genes that control the expression of tumor suppressor lncRNA via CRISPR enables cells to transcribe and translate the correct lncRNA, activating the inactivated tumor suppressor gene to achieve a therapeutic effect (Table 1).

The following are several common ways to knock out lncRNA. For shorter lncRNAs, the most effective method is to directly and completely knock out the gene that controls its expression. The designed gRNA needs to be able to cover the start and end sites of the relevant gene. Knocking out the starter or exon of the lncRNA gene is also a

ISSN 2959-409X

common method. When two or more gRNAs are used at the same time, the efficiency of CRISPR knockout will be significantly improved. In addition, the gene damage caused by this method is easier to repair. Another method is to knock out a larger fragment of the lncRNA genome. This large fragment usually contains the sequence from the last exon of the gene to the end site, that is, the 3' end of the gene (Table 1). Studies have shown that this excision method has less gene perturbation than excising the promoter and most of the exons.

3.2.2 Activation of genes of tumor suppressor lncRNAs

The target gene is typically the promoter sequence of a tumor suppressor lncRNA gene. The catalytic activity of Cas9 protein is eliminated, resulting in a dCas9 variant. The dCas9 protein promotes gene transcription. After the gRNA binds to the promoter of the tumor suppressor lncRNA gene, it recruits the transcription machinery within the cell, initiating transcription and allowing the previously inactive tumor suppressor lncRNA to be expressed.

MEG3 (Maternally Expressed Gene 3) is an important tumor suppressor lncRNA. According to Carlos D. et al., MEG3 is lost in many tumors, but when overexpressed tumor growth is significantly inhibited. It has great potential as an important target in cancer treatment, achieving a tumor suppressor effect by restoring its function [9]. Researchers have successfully activated MEG3 using CRISPR technology and demonstrated its tumor suppressor effect (Table 1).

4. Current Technical Limitations and Ethical Issues of CRISPR Technology

4.1 Limitations

Although CRISPR technology has many advantages, its current limitations and ethical issues cannot be ignored. Among them, off-target effects are one of the most serious risks. Liu suggested that when using CRISPR technology for gene editing, gRNA may bind to non-target but structurally similar gene fragments, causing the Cas9 protein to cut at the wrong site and triggering uncontrollable harmful mutations in the gene. These incorrect cuts may cause the original function of the gene to be lost, causing abnormal cell function, or even malignant genetic diseases [10]. Even if the editing is successful, the success rate is not 100%, which will cause the target protein to lose its functionality significantly and significantly reduce the therapeutic effect. How to improve the accuracy and efficiency of CRISPR while avoiding off-target effects needs to be overcome. In addition to the off-target problem, Cas9, as a foreign bacterial protein, may trigger an immune response in patients. The patient's immune system may recognize and attack it, rendering the CRISPR system unable to perform its original function. In addition, although the combination of CRISPR and CAR-T therapy has a good effect in treating blood tumors, it still has difficulties in treating solid tumors, such as insufficient efficiency and the inability of CAR-T cells to infiltrate solid tumors.

4.2 Ethical issues

This technological innovation gives rise to numerous ethical dilemmas. Human arbitrarily modify germ cell genes using this technology along with the transmission of these permanent mutations to future generations, which may spawn "superhumans" and threaten the survival of ordinary humans. Therefore, this is profound ethical questions concerning human dignity, social fairness and justice. The issue of "genetic pollution" merits attention from an ecological perspective, as genes edited via CRISPR in experiments and production processes, if spreading into the natural environment, could contaminate the natural gene pool and inflict severe ecological damage.

5. Conclusion

As an emerging gene-editing technology, CRISPR offers a completely new approach to treating intractable diseases like cancer. While this technology currently faces numerous challenges and is still in clinical trials, it holds great promise and enormous potential. For example, CRISPR-based CAR-T technology and the knockout of oncogenic lncRNAs, mentioned in this article, offer therapeutic approaches. These therapies can directly address the root causes of cancer at the genetic level, theoretically providing a radical cure that effectively avoids the risk of recurrence. Future research will need to address how to minimize off-target effects, prevent the patients' own immune system from attacking CRISPR, and further improve efficiency while reducing costs, ultimately making large-scale clinical application of CRISPR feasible.

References

[1] Aljabali A A A, El-Tanani M, Tambuwala M M. Principles of CRISPR-Cas9 technology: Advancements in genome editing and emerging trends in drug delivery. Journal of Drug Delivery Science and Technology, 2024, 92: 105338.

[2] Hao J R, Wang S X. (2021). Research Progress on the Application of CRISPR/Cas9 Technology in Disease Treatment. Journal of MuDanJiang Medical University, 2021,42(06):130-132.

[3] Niu Y M, Wang Y N, Zhang Y. Research progress of CRISPR/Cas9 technology in endometrial cancer treatment.

Modern Oncology, 2025,33(02):321-326.
[4] Xue C, Greene E C. DNA repair pathway choices in CRISPR-Cas9-mediated genome editing. Trends in Genetics, 2021, 37(7): 639-656.
[5] Wu Q, Wang S B. Progress in CRISPR/Cas9 for CAR-T cell therapy of tumors. Basic and Clinical Medicine, 2023,43(08):1313-1316.
[6] Medjebar S, Truntzer C, Perrichet A, et al. Angiotensin-converting enzyme (ACE) inhibitor prescription affects non-small-cell lung cancer (NSCLC) patients response to PD-1/PD-L1 immune checkpoint blockers. Oncoimmunology, 2020, 9(1): 1836766.
[7] Jiang W H, Gao Y S, Liu N, Zhang Y, Yang Y J, Wang G G, Dong Y H, Zhang Z M. CRISPR/Cas9 knockout of PD-1 effectively enhances the antitumor activity of EGFR-CAR-T cells against lung cancer. Chinese Journal of Gerontology, 2023,43(14):3483-3487.
[8] Li X, Hu Y, Wang Y M. CRISPR/Cas9 Technology: Exploring the Functions of IncRNAs and Their Roles in Cancer Progression. Chinese Journal of Biochemistry and Molecular Biology, 2025,41(03):364-375.
[9] Carlos D, Raquel A, Henrique C, Thiago M, Tulio F, Ana C, Mari C. Functional impact of the long non-coding RNA MEG3 deletion by CRISPR/Cas9 in the human triple negative metastatic Hs578T cancer cell line. Oncology letters, 2019, 18(6): 5941-5951.
[10] Liu J, Zheng J M. Applications and Prospects of CRISPR-Cas9 Gene Editing Technology. Chinese Journal of Chemical Education, 2025,46(04):1-8.